CLAIMS

1. A method of selecting a patient highly responsive to WT1 vaccine, comprising the following steps (a), (b) and (c):

(a) isolating a biological sample containing CTL precursor cells from a test subject;

(b) measuring the existence frequency or amount of WT1-specific CTL precursor cells in the biological sample of (a); and

(c) deciding whether or not the measured value of (b) is high by comparison with that of healthy subject, and evaluating the responsiveness to WT1 vaccine.

2. The method of selection according to claim 1, wherein the measurement of the existence frequency or amount of WT1-specific CTL precursor cells is carried out by any one of HLA monomer method, HLA dimer method, HLA tetramer method, HLA pentamer method, ELISPOT method, realtime RT-PCR technique and limiting dilution method.

3. The method of selection according to claim 2, wherein the measurement is carried out by HLA tetramer method.

4 The method of selection according to claim 3, which comprises the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTL precursor cells from a test subject;

(b) bringing an HLA tetramer comprising a WT1-derived tumor antigen peptide contact with the biological sample of (a);

(c) measuring the existence frequency or amount of WT1-specific CTL precursor cells bound to the HLA tetramer; and

(d) deciding whether or not the measured value of (c) is high by comparison with that of healthy subject, and evaluating the responsiveness to WT1 vaccine.

5. The method of selection according to claim 4, wherein the step (c) in claim 4 is carried out by measuring the proportion of HLA tetramer-bound cells among CD8-positive or CD8/CD3-positive CTL precursor cells.

6. The method of selection according to claim 4 or 5 wherein the HLA antigen as a component of HLA tetramer is an HLA-A24 antigen or an HLA-A2 antigen.

7. The method of selection according to any one of claims 4 to 6, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

8. The method of selection according to any one of claims 1 to 7, which is carried out using flow cytometry.

9. The method of selection according to any one of claims 1 to 8, wherein the responsiveness to WT1 vaccine is evaluated using as an indicator that the existence frequency or amount of WT1-specific CTL precursor cells is 1.5 times or higher compared to that of healthy subject.

10 The method of selection according to claim 1, wherein the CTL precursor cells are CTL precursor cells of effector type.

11. The method of selection according to claim 10, which uses any one of HLA monomer method, HLA dimer method, HLA tetramer method, HLA pentamer method, ELISPOT method, realtime RT-PCR technique and limiting dilution method in the measurement of the existence frequency or amount of WT1-specific CTL precursor cells of effector type.

12 The method of selection according to claim 11, which uses the HLA tetramer method.

13. The method of selection according to claim 12, which comprises the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTL precursor cells from a test subject;

(b) bringing an HLA tetramer comprising a WT1-derived tumor antigen peptide, an anti-CD8 antibody, an anti-CD45RA antibody and an anti-CD27 antibody contact with the biological sample of (a);

(c) measuring the proportion of CD45RA-postive and CD27-negative CTL precursor cells of effector type among CTL precursor cells which are positive for CD8 or CD8/CD3 and positive for binding to HLA tetramer; and

(d) deciding whether or not the measured result of (c) is high by comparison with that of healthy subject, and evaluating the responsiveness to WT1 vaccine.

14. The method of selection according to claim 13, wherein the HLA antigen as a component of HLA tetramer is an HLA-A24 antigen or an HLA-A2 antigen.

15. The method of selection according to claim 13 or 14, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

16. The method of selection according to any one of claims 10 to 15, which is carried out using flow cytometry.

17. A method of diagnosing cancer, comprising the following steps (a), (b) and (c):

(a) isolating a biological sample containing CTL precursor cells from a test subject;

(b) measuring the existence frequency or amount of WT1-specific CTL precursor cells in the biological sample of (a); and

(c) deciding whether or not the measured result of (b) is high by comparison with that of healthy subject, and evaluating whether the test subject has cancer.

18. The method of diagnosis according to claim 17, wherein the measurement of the existence frequency or amount of WT1-specific CTL precursor cells is carried out by any one of HLA monomer method, HLA dimer method, HLA tetramer method, HLA pentamer method, ELISPOT method, realtime RT-PCR technique and limiting dilution method.

19. The method of diagnosis according to claim 18, wherein the measurement is carried out by HLA tetramer method.

20. The method of diagnosis according to claim 19, which comprises the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTL precursor cells from a test subject;

(b) bringing an HLA tetramer comprising a WT1-derived tumor antigen peptide contact with the biological sample of (a);

(c) measuring the existence frequency or amount of WT1-specific CTL precursor cells bound to the HLA tetramer; and

(d) deciding whether or not the measured result of (c) is high by comparison with that of healthy subject, and evaluating whether the test subject has cancer.

21. The method of diagnosis according to claim 20, wherein the step (c) in claim 20 is carried out by measuring the proportion of HLA tetramer-bound cells among CD8-positive or CD8/CD3-positive CTL precursor cells.

22. The method of diagnosis according to claim 20 or 21, wherein the HLA antigen as a component of HLA tetramer is an HLA-A24 antigen or an HLA-A2 antigen.

23 The method of diagnosis according to any one of claims 20 to 22, wherein the WT1-derived tumor antigen peptide is selected from the

following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe(SEQ ID NO: 5).

24. The method of diagnosis according to any one of claims 17 to 23, which is carried out using flow cytometry.

25. The method of diagnosis according to any one of claims 17 to 24, wherein cancer is diagnosed using as an indicator that the existence frequency or amount of WT1-specific CTL precursor cells is 1.5 times or higher compared to that of healthy subject.

26. The method of diagnosis according to claim 17, wherein the CTL precursor cells are CTL precursor cells of effector type.

27. The method of diagnosis according to claim 26, which uses any one of HLA monomer method, HLA dimer method, HLA tetramer method, HLA pentamer method, ELISPOT method, realtime RT-PCR technique and limiting dilution method in the measurement of the existence frequency or amount of WT1-specific CTL precursor cells of effector type.

28. The method of diagnosis according to claim 27, which uses the HLA tetramer method.

29. The method of diagnosis according to claim 28, which comprises the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTL precursor cells from a test subject;

(b) bringing an HLA tetramer comprising a WT1-derived tumor antigen peptide, an anti-CD8 antibody, an anti-CD45RA antibody and an anti-CD27 antibody contact with the biological sample of (a);

(c) measuring the proportion of CD45RA-postive and CD27-negative CTL precursor cells of effector type among CTL precursor cells

which are positive for CD8 or CD8/CD3 and positive for binding to HLA tetramer; and

(d) deciding whether or not the measured value of (c) is high by comparison with that of healthy subject, and evaluating whether the test subject has cancer.

30. The method of diagnosis according to claim 29, wherein the HLA antigen as a component of HLA tetramer is an HLA-A24 antigen or an HLA-A2 antigen.

31. The method of diagnosis according to claim 29 or 30, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

32. The method of diagnosis according to any one of claims 26 to 31, which is carried out using flow cytometry.

33. A method of identifying a target molecule of WT1 vaccine said molecule being peculiar to a patient, comprising the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTL precursor cells from a test patient;

(b) applying each of plural target molecules of WT1 vaccine to the biological sample of (a);

(c) measuring the existence frequency or amount of WT1-specific CTL precursor cells in the respective biological samples of (b) and comparing the results with each other; and

(d) identifying a target molecule of WT1 vaccine effective to the test patient on the basis of the results obtained in (c).

34. The method of identification according to claim 33, wherein

the measurement of the existence frequency or amount of WT1-specific CTL precursor cells is carried out by any one of HLA monomer method, HLA dimer method, HLA tetramer method, HLA pentamer method, ELISPOT method, realtime RT-PCR technique and limiting dilution method.

35. The method of identification according to claim 34, wherein the measurement is carried out by HLA tetramer method.

36. The method of identification according to claim 35, which comprises the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTL precursor cells from a test patient;

(b) bringing each of plural HLA tetramers comprising different WT1-derived tumor antigen peptides contact with the biological sample of (a);

(c) measuring the existence frequency or amount of WT1-specific CTL precursor cells bound to the respective HLA tetramers, and comparing the results with each other; and

(d) identifying a WT1-derived tumor antigen peptide effective to the test patient on the basis of the results obtained in (c).

37. The method of identification according to claim 36, wherein the step (c) in claim 36 is carried out by measuring the proportion of HLA tetramer-bound cells among CD8-positive or CD8/CD3-positive CTL precursor cells.

38 The method of identification according to claim 36 or 37, wherein the HLA antigen as a component of HLA tetramer is an HLA-A24 antigen or an HLA-A2 antigen.

39. The method of identification according to any one of claims 36 to 38, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

40. The method of identification according to any one of claims 33 to 39, which is carried out using flow cytometry.

41. A clinical diagnostic agent for selecting a patient highly responsive to WT1 vaccine, which comprises as an ingredient an HLA monomer, an HLA dimer, an HLA tetramer or an HLA pentamer each containing a WT1-derived tumor antigen peptide.

42. The clinical diagnostic agent according to claim 41, wherein the HLA antigen as a component of an HLA monomer, an HLA dimer, an HLA tetramer or an HLA pentamer is an HLA-A24 antigen or an HLA-A2 antigen.

43. The clinical diagnostic agent according to claim 41 or 42, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

44. A kit comprising a clinical diagnostic agent according to any one of claims 41 to 43.

45. A pharmaceutical composition for treating cancer in a given patient, which comprises a target molecule identified by the method of identification of a target molecule of WT1 vaccine said molecule being peculiar to the patient according to any one of claims 33 to 40.

46. A diagnostic agent for cancer, which comprises as an ingredient an HLA monomer, an HLA dimer, an HLA tetramer or an HLA pentamer each containing a WT1-derived tumor antigen peptide.

47. The diagnostic agent according to claim 46, wherein the HLA antigen as a component of an HLA monomer, an HLA dimer, an HLA

tetramer or an HLA pentamer is an HLA-A24 antigen or an HLA-A2 antigen.

48. The diagnostic agent according to claim 46 or 47, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

49. A kit which comprises a diagnostic agent according to any one of claims 46 to 48.

50. A method of determining the suitability of a patient for WT1 vaccine, comprising the following steps (a), (b) and (c):

(a) isolating a biological sample containing CTLs from a patient after WT1 vaccine administration;

(b) measuring the existence frequency or amount of WT1-specific CTLs in the biological sample of (a);

(c) deciding whether or not the measured value of (b) is high by comparison with that of biological sample obtained before WT1 vaccine administration, and evaluating whether the patient is suitable for WT1 vaccine therapy.

51. The method of determination according to claim 50, wherein the measurement of the existence frequency or amount of WT1-specific CTLs is carried out by any one of HLA monomer method, HLA dimer method, HLA tetramer method, HLA pentamer method, ELISPOT method, realtime RT-PCR technique and limiting dilution method.

52. The method of determination according to claim 51, wherein the measurement is carried out by HLA tetramer method.

53 The method of determination according to claim 52, which comprises the following steps (a), (b), (c) and (d):

(a) isolating a biological sample containing CTLs from a patient after WT1 vaccine administration;

(b) bringing an HLA tetramer comprising a WT1-derived tumor antigen peptide contact with the biological sample of (a);

(c) measuring the existence frequency or amount of WT1-specific CTLs bound to the HLA tetramer; and

(d) deciding whether or not the measured value of (c) is high by comparison with that of biological sample obtained before WT1 vaccine administration, and evaluating whether the patient is suitable for WT1 vaccine therapy.

54. The method of determination according to claim 53, wherein the step (c) in claim 53 is carried out by measuring the proportion of HLA tetramer-bound cells among CD8-positive or CD8/CD3-positive CTLs.

55. The method of determination according to claim 53 or 54, wherein the HLA antigen as a component of HLA tetramer is an HLA-A24 antigen or an HLA-A2 antigen.

56. The method of determination according to any one of claims 53 to 55, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe(SEQ ID NO: 5).

57 The method of determination according to any one of claim 50 to 56, which is carried out using flow cytometry.

58. The method of determination according to any one of claims 50 to 57, wherein the suitability for WT1 vaccine therapy is evaluated using as an indicator that the existence frequency or amount of WT1-specific CTLs is 1.5 times or higher compared to that in the sample obtained before administration.

59. A clinical diagnostic agent for determining the suitability for WT1 vaccine which comprises as an ingredient an HLA monomer, an HLA

dimer, an HLA tetramer or an HLA pentamer each containing a WT1-derived tumor antigen peptide.

60 The clinical diagnostic agent according to claim 59, wherein the HLA antigen as a component of an HLA monomer, a HLA dimer, an HLA tetramer or an HLA pentamer is an HLA-A24 antigen or an HLA-A2 antigen.

61. The clinical diagnostic agent according to claim 59 or 60, wherein the WT1-derived tumor antigen peptide is selected from the following peptides:

Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 2),

Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 3),

Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 4) and

Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 5).

62. A kit comprising a clinical diagnostic agent according to any one of claims 59 to 61.